Telomerase reverse transcriptase promoter mutations in tumors originating from the adrenal gland and extra-adrenal paraganglia

Thomas G Papathomas¹, Lindsey Oudijk¹, Ellen C Zwarthoff¹, Edward Post¹, Floor A Duijkers², Max M van Noesel², Leo J Hofland³, Patrick J Pollard⁴, Eamonn R Maher⁵, David F Restuccia¹, Richard A Feelders³, Gaston J H Franssen⁶, Henri J Timmers⁷, Stefan Sleijfer⁸, Wouter W de Herder³, Ronald R de Krijger^{1,9}, Winand N M Dinjens¹ and Esther Korpershoek¹

¹Department of Pathology, Josephine Nefkens Institute, Erasmus MC, Rotterdam, The Netherlands

²Department of Pediatric Oncology–Hematology, Erasmus MC–Sophia Children's Hospital, Rotterdam, The Netherlands

³Sector of Endocrinology, Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands

⁴Cancer Biology and Metabolism Group, Institute of Genetics and Molecular Medicine, Edinburgh Cancer Research UK Centre, University of Edinburgh, Edinburgh, UK

⁵Department of Medical Genetics, University of Cambridge, Cambridge, UK

⁶Department of Surgery, Erasmus MC, Rotterdam, The Netherlands

⁷Division of Endocrinology, Department of Medicine, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands

⁸Department of Medical Oncology, Erasmus MC, Rotterdam, The Netherlands

⁹Department of Pathology, Reinier de Graaf Hospital, Delft, The Netherlands

Correspondence should be addressed to E Korpershoek
Email
e.korpershoek.1@erasmusmc.nl

Abstract

Hotspot mutations in the promoter of the telomerase reverse transcriptase (*TERT*) gene have been recently reported in human cancers and proposed as a novel mechanism of telomerase activation. To explore *TERT* promoter mutations in tumors originating from the adrenal gland and extra-adrenal paraganglia, a set of 253 tumors (38 adrenocortical carcinomas (ACCs), 127 pheochromocytomas (PCCs), 18 extra-adrenal paragangliomas (ea PGLs), 37 head and neck PGLs (HN PGLs), and 33 peripheral neuroblastic tumors) was selected along with 16 human neuroblastoma (NBL) and two ACC cell lines to assess *TERT* promoter mutations by the Sanger sequencing method. All mutations detected were confirmed by a SNaPshot assay. Additionally, 36 gastrointestinal stromal tumors (GISTs) were added to explore an association between *TERT* promoter mutations and SDH deficiency. *TERT* promoter mutations were found in seven out of 289 tumors and in three out of 18 human cell lines; four C228T mutations in 38 ACCs (10.5%), two C228T mutations in 18 ea PGLs (11.1%), one C250T mutation in 36 GISTs (2.8%), and three C228T mutations in 16 human NBL cell lines (18.75%). No mutation was detected in PCCs, HN PGLs, neuroblastic tumors as well as ACC cell lines. *TERT* promoter mutations preferentially occurred in a SDH-deficient setting ($P=0.01$) being present in three out of 47 (6.4%) SDH-deficient tumors vs zero out of 171 (0%) SDH-intact

Key Words

- *TERT* promoter mutations
- telomerase reverse transcriptase
- adrenocortical carcinomas
- paragangliomas
- neuroblastomas
- SDH deficiency

tumors. We conclude that *TERT* promoter mutations occur in ACCs and ea PGLs. In addition, preliminary evidence indicates a potential association with the acquisition of *TERT* promoter mutations in SDH-deficient tumors.

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Introduction

Telomerase is a ribonucleoprotein complex consisting of the telomerase reverse transcriptase (TERT) catalytic subunit and the telomerase RNA component. Telomerase is responsible for the addition of telomeric repeats at the end of linear eukaryotic chromosomes, thereby maintaining the telomere length (Mocellin *et al.* 2013). Telomeres have two major functions in normal cells (Blasco & Hahn 2003, Mocellin *et al.* 2013). First, they function to protect chromosome ends from being recognized as DNA double-strand breaks by the DNA repair machinery that can result in fusion of chromosome ends and gross chromosomal alterations. Secondly, telomeres prevent 3'-DNA shortening during cell division that can trigger cellular senescence.

In cancer cells, which display uncontrolled proliferation, maintenance of telomeres is crucial to prevent senescence induction. As a consequence, tumor cells frequently show activation of mechanisms that protect telomeres and confer cellular immortalization. In over 90% of cases, tumor cells display constitutive telomerase activation (Blasco & Hahn 2003). While there exists evidence that telomerase activity is regulated at various levels including epigenetic mechanisms (Daniel *et al.* 2012, Castelo-Branco *et al.* 2013), posttranslational modification (Li *et al.* 1998, Kang *et al.* 1999), or nuclear translocation (Liu *et al.* 2001) of TERT, upregulation of TERT at the transcriptional level, via the inappropriate binding of transcription factors such as c-myc to the core promoter region (Greenberg *et al.* 1999, Wu *et al.* 1999, Daniel *et al.* 2012), appears to be the primary mechanism yielding telomerase activation.

Consistent with this, recent studies in melanoma have demonstrated that activation of telomerase via transcriptional TERT upregulation can be caused by mutations in the core promoter region of *TERT* (Chr5) with 1 295 28 C>T, 1 295 250 C>T being the two most frequent mutation hotspots (Horn *et al.* 2013, Huang *et al.* 2013). Both mutations result in novel binding motifs for E-twenty-six transcription factors. This results in enhanced transcription of TERT, demonstrating a novel mechanism contributing to telomerase activation in human cancer

(Horn *et al.* 2013, Huang *et al.* 2013). Similarly, other studies have revealed *TERT* promoter mutations at varying site-specific frequencies in conjunctival melanoma, non-melanoma skin cancer, bladder cancer, CNS tumors, thyroid tumors, soft-tissue sarcomas, neuroblastomas (NBLs), hepatocellular carcinomas, renal cell carcinomas (RCCs), mesotheliomas, oral cavity carcinomas, and endometrial and ovarian clear cell carcinomas as well as gastrointestinal tract tumors (Arita *et al.* 2013, Brennan *et al.* 2013, Goutagny *et al.* 2013, Killela *et al.* 2013, 2014, Kinde *et al.* 2013, Koelsche *et al.* 2013, 2014, Landa *et al.* 2013, Liu *et al.* 2013a,c, Nault *et al.* 2013, Tallet *et al.* 2013, Vinagre *et al.* 2013, Griewank *et al.* 2014, Hurst *et al.* 2014, Pópulo *et al.* 2014, Qu *et al.* 2014, Scott *et al.* 2014, Wang *et al.* 2014, Wu *et al.* 2014a,b, Zhao *et al.* 2014).

The prevalence of *TERT* promoter mutations in follicular cell-derived thyroid cancer indicated that these mutations may be important in endocrine tumorigenesis (Landa *et al.* 2013, Liu *et al.* 2013a,c, Vinagre *et al.* 2013). Consistent with this prevalence, four independent research groups illustrated that more aggressive thyroid cancer subtypes were enriched for these mutations (Landa *et al.* 2013, Liu *et al.* 2013a,c, Vinagre *et al.* 2013). With regard to adrenocortical carcinomas (ACCs), a frequency of 12% has been recently shown in a single cohort (Liu *et al.* 2014). By contrast, no mutations have been observed in parafollicular cell-originated medullary thyroid carcinoma (Killela *et al.* 2013, Liu *et al.* 2013a,b,c, Vinagre *et al.* 2013), while these seem to be extremely rare genetic events in pheochromocytomas (PCCs) and paragangliomas (PGLs) (Vinagre *et al.* 2013, Liu *et al.* 2014). In the current study, we examined the presence of these mutations in tumor types originating from the adrenal gland and extra-adrenal paraganglia including ACCs, PCCs, extra-adrenal (ea)- and head and neck- (HN-) PGLs, as well as peripheral neuroblastic tumors. Given that *TERT* promoter mutations occur preferentially in specific genetic backgrounds in various tumors, any association with SDH-deficient status in PCCs, PGLs, and gastrointestinal stromal tumors (GISTs) was explored.

Subjects and methods

Tumor tissue samples and cell lines

A total of 253 formalin-fixed and paraffin-embedded (FFPE) tumors were selected, including 38 ACC samples (Erasmus MC, Rotterdam, The Netherlands: 35 primary tumors, two recurrences, and one metastasis), 127 PCCs/18 ea PGLs/37 HN PGLs (Erasmus MC, Rotterdam, The Netherlands: 167 cases; UMC St Radboud, Nijmegen, The Netherlands: 12 cases; and Birmingham, UK: three cases), and 33 peripheral neuroblastic tumors (Erasmus MC, Rotterdam, The Netherlands: 15 NBLs, eight ganglioneuromas, and ten ganglioneuromas). Tumors with mutations in the *SDH-x* genes, such as *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2*, display loss of immunohistochemical staining for SDHB (van Nederveen et al. 2009, Korpershoek et al. 2011). Given that loss of SDHB expression reflects SDH deficiency (Barletta & Hornick 2012), we will collectively use the term 'SDH deficient' for tumors displaying SDHB immunonegativity. As SDH deficiency also defines a subset of GISTs similar to the SDH-related PCC/PGL subgroup, an additional series of 36 GISTs was examined to explore the relationship between *TERT* promoter mutations and SDH deficiency in a non-endocrine tumor type.

All tumor samples were assessed anonymously according to the Proper Secondary Use of Human Tissue code established by the Dutch Federation of Medical Scientific Societies (<http://www.federa.org>). The Medical Ethical Committee of the Erasmus MC approved the study. Human NBL cell lines: SJNB-12, SJ10 (SJNB-10), SK-N-BE, KCNR, LAN-1, LAN-5, N206, NGP-C4, NMB, TR-14, SH-EP-2/tet2, SJ1 (SJNB-1), SK-N-SH, SH-SY5Y, GI-ME-N, and SK-N-AS as well as human ACC cell lines NCI-H295 (source: ATCC (CRL-2128); method of authentication: STR profiling; passage number: P7) and SW13 (source: ATCC (CCL-105); method of authentication: STR profiling; passage number: P2) were also included in the analysis. The NBL cell lines have been originally obtained from the NCI and are molecularly well characterized/established in the field of NBL research (Thiele 1998). These cell lines were grown from the original clones and used after <35 passages; all have been checked for molecular characteristics in our departmental research laboratory.

DNA isolation and *TERT* promoter mutation analysis

DNA isolation from tumors was carried out using standard procedures following manual microdissection of all

tumor samples to ensure a >80% neoplastic cell content. Standard PCR was performed to amplify a 163 bp fragment of the *TERT* promoter region, covering all previously described mutations (C228T, CC229TT, CC242TT, and C250T, corresponding to nucleotide positions -124, -125, -138, and -145 from the translational start site (UCSC: chr5 nt 1 295 104)), using forward primer 5'-GTCCTGCCCTTCACCTT-3' and reverse primer 5'-CAGCGCTGCCTGAACTC-3'. Subsequently, PCR products were used as templates for direct sequencing using the BigDye Terminator V3.1 cycle sequencing kit (Applied Biosystems). Products were analyzed on the ABI Prism 3130 Genetic Analyzer (Applied Biosystems).

TERT promoter mutations were confirmed by a SNaPshot assay using the ABI Prism SNaPshot Multiplex Kit (Applied Biosystems) as described previously (Allory et al. 2014). In brief, after the multiplex SNaPshot reaction, the products were treated with shrimp alkaline phosphatase to remove excess dideoxynucleotide triphosphates, and subsequently were labeled and separated in a 25-min run on 36-cm-long capillaries in an automatic sequencer (ABI Prism 3130 Genetic Analyzer, Applied Biosystems). GeneScan Analysis Software, version 3.7 (Applied Biosystems) was used for data analysis. All experimental conditions are available on request. Probe sequences of the SNaPshot reaction are given in Supplementary Table 1, see section on supplementary data given at the end of this article.

SDHB/SDHA immunohistochemistry, mutation screening, and loss of heterozygosity analysis

SDH (immunohistochemistry (IHC) and/or mutation) status was known for 218 PCCs, ea PGLs, HN PGLs, and GISTs. To investigate the SDH status of the ACC samples included in the current study, these samples were arranged in a tissue microarray (TMA) format along with additional adrenocortical adenomas (ACAs), normal adrenal tissue, and control tissue samples (38 ACC, 17 ACA, five normal adrenal tissue, and 12 control tissue samples) using an automated TMA constructor (ATA-27 Beecher Instruments, Sun Prairie, WI, USA) available at the Department of Pathology, Erasmus MC. For each tumoral case, representative areas were selected and marked on a hematoxylin and eosin-stained slide. Accordingly, two tissue cores with a diameter of 1 mm were extracted from 'donor' block and brought into the 'recipient' paraffin block at predefined coordinates. SDHA and SDHB immunostaining procedures were performed on 4–5 µm TMA sections with a mouse monoclonal Ab14715 antibody

(Mitosciences, Abcam, Cambridge, UK; 1:500 dilution) against SDHA and a rabbit polyclonal HPA002868 antibody (Sigma–Aldrich Corp., St. Louis, MO, USA; 1:400 dilution) against SDHB on an automatic Ventana Benchmark Ultra System (Ventana Medical Systems, Inc., Tuscon, AZ, USA). If the internal control (granular staining in endothelial cells) was positive, slides were considered as informative. From SDHB-immunonegative/SDHA-immunopositive ACCs, i) the entire *SDHA*, *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* coding sequences were assessed at the germline and somatic levels for mutations using an Ion AmpliSeq Custom Panel that was sequenced on the Ion Torrent Personal Genome Machine (PGM; Life Technologies) on 10 ng FFPE tumor DNA according to the manufacturer's protocols. In short, libraries were made using the Ion AmpliSDefault 2.0 Library Kit. Template was prepared using the Ion OneTouch Template Kit and sequencing was performed with the Ion PGM Sequencing 200 Kit v2.0 on an Ion 316v2 chip. Data were analyzed using the Torrent Suite Software, version 3.6.2 (Life Technologies). Annotation of variant calls was performed with Annovar (<http://www.openbioinformatics.org/annovar/>; Wang *et al.* 2010) and facilitated using an in-house galaxy platform/server on which Annovar wrapper was installed (Giardine *et al.* 2005, Blankenberg *et al.* 2010, Goecks *et al.* 2010, Hiltmann *et al.* 2014). The variants with a read frequency higher than 10%, not known as common polymorphisms according to 1000G2012 April and ESP6500, non-synonymous with a minimum of five forward/reverse variant reads and 100 total depth reads were retained as interesting ones (mutations) (sequences of all primers and probes are available upon request); and large intragenic deletions using multiplex ligation-dependent probe amplification (MLPA) assay were analyzed using a commercially available kit (SALSA MLPA P226-B2; MRC Holland, Amsterdam, The Netherlands) and ii) loss of heterozygosity (LOH) analysis was performed for polymorphic microsatellite markers flanking the *SDHB*, *SDHC*, *SDHD*, and *SDHAF2* genes as described previously (Papathomas *et al.* 2013).

RNA extraction and *TERT* expression analysis by quantitative real-time PCR

Total mRNA was extracted from human primary adrenal tissue (one ACC harboring a *TERT* promoter mutation, two ACCs without *TERT* promoter mutation, one ACA, and two normal adrenocortical tissue samples) or cell pellets (HEK and SW13 cell lines) using TRIzol reagent (Invitrogen Life Technologies) and the RNA-containing

supernatant was purified using RNeasy spin columns (Qiagen Benelux B.V.). First-strand cDNA synthesis was performed on 200 ng total RNA using qScript cDNA Supermix (Quanta Biosciences, Gaithersburg, MD, USA), followed by *TERT*-specific and hypoxanthine phosphoribosyltransferase 1 (*HPRT1*)-pre-amplification using PerfeCTa PreAmp SuperMix (Quanta Biosciences). The PreAmp product was diluted and used to assess human telomerase expression in all samples by quantitative real-time PCR in triplicate using TaqMan (Applied Biosystems) gene expression assays. *TERT* (*TERT* Hs00972656_m1) was measured relative to *HPRT* (*HPRT1*) expression. The relative amount of RNA was calculated by the $2^{-\Delta\Delta CT}$ method. Fold changes in gene expression were determined by comparing expression levels of tumor tissue or cell lines with normal adrenocortical tissue. No RNA was available to test the remaining tumors endowed with the *C228T* and *C250T* mutations.

Statistical analysis

Statistical analysis was performed using SPSS (IBM SPSS Statistics, version 20) on a series of 218 tumors (PCCs/PGLs/GISTs) of known SDH status. Fisher's exact test was used to determine the relationships between the presence of a *TERT* promoter mutation and SDH deficiency. Statistical differences were considered to be significant when the *P* value is <0.05.

Results

Prevalence of *TERT* promoter mutations in various human tumors and cell lines

TERT promoter mutations were found in seven out of 289 tumors investigated with *C228T* being the most frequent substitution. There were four *C228T* mutations in 38 ACCs (10.5%), two *C228T* mutations in 18 ea PGLs (11.1%), and one *C250T* mutation in 36 GISTs (2.8%). Clinicopathological and genetic data of these patients are given in Table 1 in detail, while representative somatic *TERT* promoter mutations (*C228T* and *C250T*) detected both by the Sanger sequencing method and a SNaPshot assay are displayed in Fig. 1. Out of seven, six *TERT* promoter-mutated tumors were metastatic (Table 1). Although three out of four mutation-positive ACCs were characterized by highly aggressive biological behavior, we could not perform proper survival analysis due to the limited number of these cases. Mutations were not detected in any of the 127 PCCs, 37 HN PGLs, and 33 peripheral neuroblastic tumors.

Table 1 Clinicopathological and genetic data of patients with *TERT* promoter-mutated tumors

Case no.	Tumor type	Anatomic site	Sex	Age	<i>TERT</i> promoter mutation	<i>SDH</i> -deficient	Weiss score	Metastatic disease/site	Follow-up/status
1	ACC	Adrenal gland	F	50	<i>C228T</i>	No	5	Yes/liver	9 mo/DOD
2	ACC	Adrenal gland	M	51	<i>C228T</i>	No	6	Yes/liver, lung, and bone	12 mo/DOD
3	ACC	Adrenal gland	M	42	<i>C228T</i>	Yes	8	Yes/liver, lung, and LNs	2 mo/DOD
4	ACC	Adrenal gland	F	58	<i>C228T</i>	No	7	None	105 mo/AWED
5	ea PGL	Urinary bladder	M	46	<i>C228T</i>	Yes ^a	–	Yes/LNs	NA
6	ea PGL	Urinary bladder	M	61	<i>C228T</i>	Yes ^b	–	Yes/LNs	226 mo/AWED
7	GIST	Stomach	F	57	<i>C250T</i>	Yes ^c	–	Yes/liver	33 mo/DOD

ACC, adrenocortical carcinoma; AWED, alive without evidence of disease; DOD, dead of disease; ea PGL, extra-adrenal paraganglioma; GIST, gastrointestinal stromal tumor; LN, lymph nodes; NA, not available.

^aSDHB IHC–/SDHA IHC– as previously published in Korpershoek et al. (2011) (non-informative on mutational analysis due to poor DNA quality).

^bSDHB IHC–/SDHA IHC+ (*SDHB* c.292T>C p.Cys98Arg).

^cSDHB IHC–/SDHA IHC+ (*SDHD* c.416T>C p.Leu139Pro).

The *TERT* promoter mutation *C228T* was found in three out of 16 (18.8%) human NBL cell lines (SJNB-10, SJNB-12, and SK-N-BE), while no mutations were present in the two ACC cell lines (Supplementary Table 2, see section on supplementary data given at the end of this article).

Enrichment of *TERT* promoter mutations in *SDH*-deficient tumors

Given that a subset of PCCs, PGLs, and GISTs is associated with germline *SDH-x* mutations and/or loss of SDHB immunoreexpression (collectively known as SDH-deficient tumors) and three out of 47 (6.4%) SDH-deficient tumors harbored a *TERT* promoter mutation, we analyzed the relationship between the SDH-deficient status and the

presence of *TERT* promoter mutations. It has been demonstrated that *TERT* promoter mutations occur preferentially in SDH-deficient tumors (6.4 vs 0%; $P=0.01$).

Loss of SDHB expression in *TERT* promoter-mutated ACCs

Out of 55 adrenocortical tumor samples, one ACC harboring a *TERT* *C228T* mutation was SDHB immunonegative/SDHA immunopositive. SDHB/SDHA IHC was re-performed on whole-tissue sections in all four *TERT* promoter-mutated ACCs and accordingly confirmed the aforementioned finding. Mutational analysis did not reveal any pathogenic germline or somatic *SDHB/C/D/AF2* mutations, while large intragenic *SDHB*, *SDHD*, and *SDHAF2* deletions were detected only at the somatic

Figure 1

Somatic *TERT* promoter mutations in ACC2 and G104 as detected using the Sanger sequencing method (upper panel) and confirmed using a SNaPshot assay (lower panel). Arrows in the upper panel indicate the *C228T* and

C250T mutations as displayed in the sequencing chromatograms (from left to right), while arrows in the lower panel indicate the same mutations in the SNaPshot electropherograms.

Figure 2

Quantitative real-time *TERT* expression analysis in human HEK and SW13 cell lines, normal adrenocortical tissues (NATs), adrenocortical adenomas (ACAs), and adrenocortical carcinomas (ACCs) with or without *TERT* promoter mutations. *TERT* expression was measured relative to the housekeeping *HPRT* gene with fold changes normalized to expression in human adrenocortical tissue for all samples.

level. Being consistent with the latter, LOH analysis revealed LOH both at the *SDHAF2* and *SDHD* loci and for a microsatellite marker telomeric to *SDHB* gene.

Role of *TERT* promoter mutation in gene expression

To determine as to whether this mutation resulted in increased *TERT* expression, quantitative RT-PCR was performed on a single *TERT* promoter-mutated ACC for which frozen material was available. Significant *TERT* expression was detected in the promoter-mutated ACC, while the non-mutated ACCs demonstrated very low to negligible *TERT* expression similar to that detected in normal adrenocortical tissue as shown in Fig. 2. *TERT* expression in the *TERT* promoter-mutated ACC was approximately half that of the control HEK and SW13 cell lines.

Discussion

TERT promoter mutations have recently been shown as a novel genetic mechanism underlying telomerase activation and present in diverse human tumors with the highest frequencies in bladder cancer, CNS tumors, melanomas, hepatocellular carcinomas, and myxoid liposarcomas (Arita et al. 2013, Horn et al. 2013, Huang et al. 2013, Killela et al. 2013, Kinde et al. 2013, Landa et al. 2013, Liu et al. 2013a,c, Nault et al. 2013, Tallet et al. 2013, Vinagre et al. 2013, Allory et al. 2014, Hurst et al. 2014, Koelsche et al. 2014). In this study, we expanded the spectrum of *TERT* promoter-mutated tumors to ACCs,

ea PGLs, and GISTs, while adding *TERT* promoter mutations to other mechanisms of *TERT* mRNA upregulation in adrenocortical tumorigenesis (Else et al. 2008, Else 2009, Liu et al. 2014) consistent with previously reported associations in other tumor types (Arita et al. 2013, Nault et al. 2013, Tallet et al. 2013, Vinagre et al. 2013).

Interestingly, we found that two ea PGLs of urinary bladder harboring *TERT* promoter mutations were SDH-deficient tumors. Other tumors that have been linked to SDH deficiency are GISTs (Barletta & Hornick 2012). To further explore a potential association between the presence of these mutations and SDH deficiency, a series of 36 GISTs were examined and subsequently revealed one *SDHD*-mutated GIST containing a *TERT* promoter mutation. This prompted us to examine the SDH status of the *TERT* promoter-mutated ACCs. Despite the fact that this latter tumor type has never been associated with SDH deficiency, we showed loss of SDHB expression in one of the aforementioned ACCs, but without any germline *SDH-x* pathogenic mutations or gross deletions detected. This finding further extends the spectrum of tumors displaying loss of SDHB and/or SDHA expression in the absence of causative *SDH-x* mutations, including a clinicopathologically and biologically distinctive subset of KIT/PDGFRA WT GISTs (Barletta & Hornick 2012, Nannini et al. 2013), poorly and/or un-differentiated NBLs (Feichtinger et al. 2010), and a clear cell RCC with sarcomatous dedifferentiation (Papathomas et al. 2013).

Although only a small subset of SDH-deficient ea PGLs and GISTs harbored a *TERT* promoter mutation, the latter did occur exclusively in the SDH-deficient setting. As all SDH-deficient *TERT* promoter-mutated tumors were clinically aggressive, these observations may reflect that *TERT* promoter mutations can cooperate in SDH-deficient cells to support an enhanced tumor progression. Whether or not the latter could be attributed to telomerase-mediated extension of telomeres extending the lifespan of mutated clones, conferring them infinite proliferation potential as well as enabling the accumulation of additional genetic alterations, and/or to other non-canonical functions interfering with extra-telomeric tumor-promoting pathways remains to be elucidated (Greider & Blackburn 1985, Cao et al. 2002, Stewart et al. 2002, Choi et al. 2008, Parkinson et al. 2008, Park et al. 2009, Martinez & Blasco 2011, Mukherjee et al. 2011, Liu et al. 2013b).

Similarly, a selective combinatorial genetic alteration pattern has been highlighted in various tumor types (Arita et al. 2013, Horn et al. 2013, Killela et al. 2013, 2014, Landa et al. 2013, Liu et al. 2013a, Tallet et al. 2013,

Vinagre *et al.* 2013, Heidenreich *et al.* 2014, Pópulo *et al.* 2014, Wu *et al.* 2014b). In CNS tumors, *TERT* promoter mutations mostly occur in i) tumors with EGFR amplification, ii) *IDH* WT tumors, iii) almost all tumors with concurrent total chromosome 1p and 19q loss and *IDH1/IDH2* mutations, and iv) *IDH1/IDH2*-mutated tumors exhibiting oligodendroglial morphologies (Arita *et al.* 2013, Killela *et al.* 2013, 2014). Similar to the previously reported coexistence with *BRAF*-activating mutations or with concomitant *BRAF* and *CDKN2A* alterations in melanomas (Horn *et al.* 2013, Heidenreich *et al.* 2014, Pópulo *et al.* 2014), two independent groups displayed a preferential occurrence of *TERT* promoter mutations in *BRAF* V600E mutation-positive papillary thyroid carcinomas (Liu *et al.* 2013a, Vinagre *et al.* 2013), while Landa *et al.* (2013) observed a significant co-occurrence of *TERT* mutations with *BRAF* and *RAS* mutations in poorly differentiated thyroid carcinomas and anaplastic thyroid carcinomas. In bladder cancer and mesotheliomas, *TERT* promoter mutations were frequently associated with inactivating mutations in the TP53/RB1 signaling pathway (Wu *et al.* 2014b) and tumor suppressor *CDKN2A* gene inactivation respectively (Tallet *et al.* 2013), while a significant co-occurrence with *CTNNB1*-activating mutations has been reported in hepatocellular carcinomas and adenomas with malignant transformation (Nault *et al.* 2013, Pilati *et al.* 2014).

In this study, all *TERT* promoter-mutated tumors except one appeared to be metastatic (Table 1); this being in accordance with previous studies demonstrating that these mutations are more highly prevalent in advanced forms of particular malignancies, including follicular cell-derived thyroid cancer, melanoma, and primary glioblastoma (Horn *et al.* 2013, Killela *et al.* 2013, Landa *et al.* 2013, Liu *et al.* 2013a,c, Vinagre *et al.* 2013). By contrast, *TERT* promoter mutations occur as an early genetic event in bladder tumorigenesis (Kinde *et al.* 2013, Allory *et al.* 2014, Hurst *et al.* 2014), meningiomas prone to malignant progression (Goutagny *et al.* 2013), as well as in *CTNNB1*-mutated hepatocellular adenomas associated with the last step of the adenoma–carcinoma transition (Nault *et al.* 2013, Pilati *et al.* 2014). In this context, *BRAF* V600E-mutated papillary thyroid carcinomas, which are more aggressive than their *BRAF* WT counterparts (Liu *et al.* 2013a), are preferentially enriched for *TERT* promoter mutations (Liu *et al.* 2013a, Vinagre *et al.* 2013).

TERT promoter mutations seem to be present in NBLs at low frequencies (~9%; two out of 22; Killela *et al.* 2013). NBLs are characterized by high expression and/or amplification of NMYC, the neuronal equivalent of c-myc.

A direct binding of NMYC to the *TERT* promoter has not been established. In this study, *TERT* promoter mutations were not detected in any peripheral neuroblastic tumor being consistent with the data stemming from a recent whole-genome sequencing project for NBLs (Molenaar *et al.* 2012) and similar observations concerning other pediatric embryonal tumors, such as a clinically distinct molecular subtype of medulloblastoma (Killela *et al.* 2013, Koelsche *et al.* 2013, Remke *et al.* 2013). Nevertheless, three human NBL cell lines harbored *TERT* promoter mutations indicating that lack in tumor samples could be attributed either to decreased sensitivity of the technique owing to the presence of normal cells or to the inclusion of other peripheral neuroblastic tumor types, such as ganglioneuroblastomas and/or ganglioneuromas.

In summary, this study demonstrates that *TERT* promoter mutations occur, albeit rarely, in tumors originating from the adrenal cortex (ACCs) and paraganglia of urinary bladder. Their absence in PCCs and HN PGLs indicates that these seem unlikely to be critical genetic events in their development and/or progression. In addition, it provides preliminary evidence of a potential association with the acquisition of *TERT* promoter mutations in a subset of aggressive SDH-deficient tumors. Further studies are warranted to elucidate this connection and to provide mechanistic insights into the effects of these gain-of-function mutations at the *TERT* promoter on SDH-*x*-related tumorigenesis as well as their prognostic relevance in SDH-related tumor types.

Supplementary data

This is linked to the online version of the paper at <http://dx.doi.org/10.1530/ERC-13-0429>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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